

**OOMYCETE FTSZ-MT AS A TARGET FOR
ANTIMICROBIAL-SPECIFIC BIOCIDES**

5

Field of the Invention

The invention relates to the field of antimicrobials, and more specifically to isolated polynucleotides encoding FtsZ proteins as targets for screening methods to obtain antimicrobials specific for oomycetes and α -proteobacteria.

10

Background of the Invention

Oomycetes

15

The Oomycota are filamentous protists which gain their nutrition by absorbing food from surrounding water or soil, or by invading the body of another organism to feed on fluids there. There are more than 500 species in the Oomycota, including the so-called water molds and downy mildews. As such, oomycetes play an important role in the decomposition and recycling of decaying matter. Parasitic species have had a impact on human activities by destroying crops or fish.

20

“Oomycota” means “egg fungi,” which refers to the large round oogonia, or structures containing the female gametes. Oomycetes are oogamous, producing large non-motile gametes called eggs, and smaller gametes called sperm. The Oomycota have a very sparse fossil record. A possible oomycete has been described from Cretaceous amber.

25

The Oomycota were once classified as fungi, because of their filamentous growth, and because they feed on decaying matter as do fungi. The cell wall of oomycetes is not, however, composed of chitin as in the fungi, but instead is made up of a mix of cellulosic compounds and glycan. Another distinguishing feature is that the nuclei within the filaments are diploid, with two sets of genetic information, not haploid as in the fungi.

30

The ultrastructure, biochemistry, and molecular sequences of these organisms indicate that they belong with the Chromista. The free-swimming spores which are produced bear two dissimilar flagella, one with mastigonemes, a feature that is common

in the chromists, as is the presence of the chemical mycolaminarin, an energy storage molecule similar to those found in kelps and diatoms. Thus, oomycetes are classified as belonging to the taxonomic minority known as heterotrophic chromists.

5 Some oomycetes, also called molds or water molds, are parasites on other organisms. Water molds may grow on the scales or eggs of fish, or on the skins of amphibians. The water mold *Saprolegnia* causes lesions on fish which cause problems when the water is stagnant as is often the case in aquaria or fish farms, or at high population densities such as when salmon swim upstream to spawn. *Saprolegnia* can spread rapidly, damaging a large surface area. These infections can be difficult to treat.

10 Other species of *Saprolegnia* are parasitic on aquatic invertebrates such as rotifers, nematodes, and arthropods, and on diatoms.

Their greatest impact on humans, however, comes from the many species of oomycete which are parasites on flowering plants. These include root rotting oomycetes, seedling damping mold, blister rusts, white rusts *Albugo*, and the downy mildews that affect grapes, lettuce, corn, cabbage, and many other crop plants. Two of these disease-causing oomycetes have had a major impact on world history.

15

The first of these is *Phytophthora infestans*, the organism which causes late blight of potato. The potato is native to South America, but after it was introduced to Europe in the late 16th century, it quickly became an important food crop. Late blight did not follow its host plant across the Atlantic until much later. The disease organism grows into the stem and leaf tissues, causing death, and may also infest the tubers. The disease spreads rapidly under cool and damp conditions, which are common in western Europe. In one famous case, in just one week during the summer of 1846, this disease wiped out almost the entire potato crop of Ireland, where potatoes were the primary food of the poor. This *Phytophthora* blight caused the deaths of nearly a million inhabitants of Ireland, and precipitated the emigration of an additional 1.5 million to other countries. Other species of *Phytophthora* destroy eucalyptus, avocado, pineapples, and other tropical crop plants. While chemicals have been developed to combat oomycete infections, the emergence of chemical-resistant strains combined with banning of effective chemicals has combined to create a *P. infestans* epidemic which is now a serious problem.

20

25

30

09770509.01304
The other oomycete which has severely impacted recent history is *Plasmopara viticola*, the downy mildew of grapes. It is a native of North America, but in the late 1870s was accidentally introduced to Europe. At the time, the French wine industry was concerned over a massive aphid infestation, and so brought resistant vine strains over from America to breed them into their own grapes. When these American stocks arrived, the American vines also brought the downy mildew, which almost wiped out the entire French wine industry. The industry was saved by the serendipitous discovery of Bordeaux mixture, a mixture of lime and copper sulfate, which brought the disease under control when applied to the leaves of the plants. This discovery is also important for being the first known fungicide, and in fact the first chemical used to control a plant disease. However, Bordeaux mixture is hazardous to many other organisms.

A current problem is Sudden Oak Death Syndrome which is caused by a previously unknown species of *Phytophthora*. First observed in 1995, within 5 years the infestation by this plant pest has spread 350 miles along the California coast infecting tan oaks, coast live oaks and black oaks. In some areas, as many as 80% of the trees are infected. A state of emergency has been declared in Marin County, one of the hardest-hit areas. Effective, environmentally-safe means to combat this *Phytophthora* species have not been determined.

This disease not only impacts oaks, but also the thousands of animal species that rely on leaves and acorns from these trees, as well as increasing the fire risk posed by the rapid accumulation of dead trees.

FtsZ proteins

FtsZ (named after filamenting temperature sensitive strain Z) is a 40 kDa protein ubiquitous in Eubacteria and Archaea. The bacterial cell division protein FtsZ is a key component of the bacterial cell division machinery. Fusion constructs of FtsZ with green-fluorescent protein have shown that, at the onset of division, FtsZ forms a filamentous ring at the site of cell division, and disassembles after septation is complete. FtsZ can self-assemble into rafts of long filaments having curving edges, as well as into sheets and rings. A cytoskeletal role for FtsZ has been postulated based on its ability to undergo GTP-dependent polymerization *in vitro* and its similarity to tubulin. Bacterial FtsZ shares limited sequence identity with tubulin, and the axial repeat of these

filaments is around 40 Å, the same as that of tubulin monomers in a protofilament. The structure of FtsZ has been solved by X-ray crystallography using crystals obtained from the FtsZ1 protein from the hyperthermophilic methanogen *Methanococcus jannaschii*. The model, refined to 2.8 Å, includes a molecule of GDP.

5 Until recently, the only known eukaryotic FtsZs were chloroplastic FtsZs (FtsZ-cp) in plants. In higher plants, the FtsZ protein is involved in plastid division, but there is little information on its involvement in the plastid-dividing apparatus. Comparison of several prokaryotic and eukaryotic FtsZ proteins shows that there are six highly conserved domains in the core region of FtsZ. Phylogenetic analysis indicates that
10 *Cyanidium caldarium* RK-1 and other eukaryotic FtsZ genes are the descendants of cyanobacterial FtsZ genes, supporting the current agreement that FtsZ is involved in plastid division. Expression studies of the gene encoding FtsZ (the FtsZ gene) in *C. caldarium* indicated that the FtsZ gene is transcribed just before plastid division. Eukaryotic FtsZ isolated from *Arabidopsis thaliana* contains a glycine-rich tubulin
15 signature motif which is conserved among FtsZ proteins and tubulins, and which is important for GTP binding, which further supports the suggestion that eukaryotic FtsZ proteins may have a cytoskeletal role analogous to that of tubulin. (U.S. Patent No. 5,981,836).

20 In major groups of eukaryotes, such as animals, plants and true fungi, mitochondrial division is mediated by a non-FtsZ mechanism. There are no FtsZ genes in yeast or nematode where the respective genomes have been completely sequenced. The recent discovery of the mitochondrial form of FtsZ (FtsZ-mt) in a chromophyte alga (Beech *et al.* (2000) *Science* 287: 1276-1279) strongly suggests that in primitive eukaryotes, unlike major groups of eukaryotes, FtsZ-mt is required for mitochondrial
25 division.

Summary of the Invention

30 The invention is directed to methods for identifying and using compounds having antimicrobial activity, methods for identifying and using compounds having anti-FtsZ activity, methods for suppressing microbial growth, methods of crop improvement, methods of producing an FtsZ-mt protein, methods of using a virtual

screen to identify potential inhibitors of FtsZ-mt proteins which do not inhibit FtsZ-cp proteins, and methods of treating fish infected with an oomycete pathogen. The invention further provides compounds identified by the methods of the present invention and isolated DNA molecules encoding mitochondrial FtsZ proteins, preferably from oomycetes.

Embodiments of the present invention provide a method for identifying compounds having antimicrobial activity by combining at least a portion of a polypeptide or protein which includes an amino acid sequence encoding an oomycete FtsZ-mt protein with a compound to be tested for the ability to bind to an FtsZ-mt protein or to the polypeptide, under conditions conducive to binding, selecting a compound thus identified as capable of binding to the FtsZ-mt protein or the polypeptide, applying the selected compound to a microbe to test for antimicrobial activity, and then selecting compounds having antimicrobial activity. These methods may be practiced using FtsZ-mt protein encoded by an isolated DNA molecule having a nucleotide sequence substantially similar to the sequence of SEQ ID NO: 1, or SEQ ID NO: 3, or SEQ ID NO: 5, or SEQ ID NO: 9. These methods may be practiced using FtsZ-mt protein encoded by an isolated DNA molecule which encodes the amino acid sequence of SEQ ID NO: 2, or SEQ ID NO: 4, or SEQ ID NO: 10. In one embodiment, the microbe is an oomycete such as *Phytophthora infestans*. A further aspect of the present invention is a compound identified by practicing methods of the present invention for identifying compounds having antimicrobial activity.

Embodiments of the present invention provide a method for identifying an inhibitor of FtsZ-mt activity having antimicrobial activity by combining an FtsZ-mt protein or a portion thereof, and a compound to be tested for the ability to inhibit the activity of the FtsZ-mt protein or an active region thereof, under conditions conducive to such inhibition, selecting a compound thus identified as capable of inhibiting said FtsZ-mt activity, applying the compound identified as capable of inhibiting said FtsZ-mt activity to a microbe to test for antimicrobial activity, and selecting compounds having antimicrobial activity. Methods of the present invention for identifying an inhibitor of FtsZ-mt activity having antimicrobial activity may be practiced using at least a portion of an FtsZ-mt protein or a substantially similar polypeptide, encoded by

an isolated DNA molecule having a nucleotide sequence substantially similar to the sequence of SEQ ID NO: 1, or SEQ ID NO: 3, or SEQ ID NO: 5, or SEQ ID NO: 9. Methods of the present invention for identifying an inhibitor of FtsZ-mt activity having antimicrobial activity may be practiced using FtsZ-mt protein or a portion thereof, or a substantially similar polypeptide, encoded by an isolated DNA molecule which encodes an amino acid sequence substantially similar or identical to at least a portion of SEQ ID NO: 2, or SEQ ID NO: 4, or SEQ ID NO: 10. In one embodiment of the method for identifying an inhibitor of FtsZ-mt activity having antimicrobial activity, the microbe is an oomycete such as *Phytophthora infestans*. A further aspect of the present invention is a compound identified by the method of the present invention for identifying an inhibitor of FtsZ-mt activity having antimicrobial activity.

Embodiments of the present invention further provide methods for suppressing oomycete growth by applying to an oomycete a compound that inhibits the activity of an oomycete FtsZ-mt protein including an amino acid sequence encoded by a nucleotide sequence substantially similar to a polynucleotide selected from, for example, SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 or SEQ ID NO: 9, in an amount sufficient to suppress growth of the oomycete.

Embodiments of the present invention provide a method of crop improvement by applying to a plant, plant part, plant seed, or surrounding soil a compound having antimicrobial activity identified by methods of the present invention for identifying compounds having antimicrobial activity, where the compound is applied in an amount that inhibits the growth of microbes of at least one microbe taxon without significantly suppressing the growth of the plant or plant seed. Another object is a method of crop improvement by applying to a plant, plant part, plant seed, or surrounding soil a compound having antimicrobial activity identified by methods of the present invention for identifying an inhibitor of FtsZ-mt activity having antimicrobial activity, where the compound is applied in an amount that inhibits the growth of microbes of at least one microbe taxon without significantly suppressing the growth of the plant or seed. Further aspects of methods of crop improvement in accordance with the present invention include microbes of various taxa, such as, for example, *Lagenaria*, *Peronophythora*, *Trachysphaera*, *Pythium*, *Phytophthora*, *Albugo*, *Peronospora*,

09770509-013901
Plasmopora, Pseudoperonospora, Bremia, Bremiella, Basidiophora and the like. Yet further aspects of methods of crop improvement in accordance with the present invention include treatment of any crop plant, such as, for example, potato, tomato, tobacco, oaks, coffee berry (*Rhamnus* sp.), squash, cotton, sorghum, peas, onion, melon, cucumber, peas, beets, watermelon, peppers, Port Orford Cedar, taro, apple, *Brassica* species, sweet potato (*Ipomea*), spinach, beans, grapevine, sunflower, hops, lettuce, violets, asters, soybeans, and cereals including maize, rice, barley, wheat, rye, and the like.

Embodiments of the present invention provide an isolated DNA molecule which encodes a mitochondrial FtsZ protein obtained from an oomycete. In one embodiment, the isolated DNA molecule is obtained from *Phytophthora infestans*. In other embodiments, the isolated DNA molecule obtained from an oomycete has the sequence of SEQ ID NO: 1, or SEQ ID NO: 3, or SEQ ID NO: 5, or SEQ ID NO: 9.

Embodiments of the present invention provide an isolated DNA molecule capable of hybridizing to a polynucleotide having a sequence selected from, for example, SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, or SEQ ID NO: 9, wherein the hybridization takes place in 7% sodium dodecyl sulfate, 0.5 M NaPO₄, 1 mM EDTA at 50°C for a pre-determined time followed by washing in 2 X SSC, 0.1% sodium dodecyl sulfate at 50°C. Further aspects of the invention provide an isolated DNA molecule having at least 65% sequence identity with the polynucleotide sequence of, for example, SEQ ID NO: 1, or SEQ ID NO: 3, or SEQ ID NO: 5, or SEQ ID NO: 9.

Other aspects of the invention provide an isolated DNA molecule which encodes a peptide having an amino acid sequence selected from, for example, SEQ ID NO: 2, SEQ ID NO: 4, or SEQ ID NO: 10. Further aspects of the invention provide an isolated peptide having a sequence such as SEQ ID NO: 2, SEQ ID NO: 4, or SEQ ID NO: 10.

Embodiments of the present invention provide methods of producing at least one FtsZ-mt protein by transforming a suitable host cell with a construct including an isolated polynucleotide encoding an oomycete FtsZ-mt protein, culturing the host cells under conditions in which the cells express said isolated polynucleotide, and recovering the oomycete FtsZ-mt protein. Embodiments of these methods include producing FtsZ-mt protein by transforming a suitable host cell with an expression vector including an

isolated polynucleotide encoding an oomycete FtsZ-mt protein. In various embodiments, the isolated polynucleotide has a sequence selected from, for example, SEQ ID NO: 1, SEQ ID NO:3, SEQ ID NO: 5, or SEQ ID NO: 9.

Embodiments of the present invention further provide methods of using a virtual screen to identify potential inhibitors of FtsZ-mt proteins which do not inhibit FtsZ-cp proteins, by predicting the three dimensional structure of an FtsZ-mt protein, predicting the three-dimensional structure of an FtsZ-cp protein, and using a computer model to identify molecules which bind to FtsZ-mt proteins but not to FtsZ-cp proteins as potential inhibitors of FtsZ-mt proteins. In some embodiments, the FtsZ-mt protein is an oomycete FtsZ-mt protein. Preferably, the oomycete belongs to the genus *Phytophthora*. Even more preferably, the oomycete is *Phytophthora infestans*. Preferably, the FtsZ-mt protein has at least about 20 amino acids of the sequence of any of SEQ ID NO: 2, SEQ ID NO: 4, or SEQ ID NO: 10. Also preferably, the FtsZ-mt protein has a sequence selected of any of SEQ ID NO: 2, SEQ ID NO: 4, or SEQ ID NO: 10. Another aspect is an inhibitor of FtsZ-mt proteins identified by the method of using a virtual screen to identify potential inhibitors of FtsZ-mt proteins which do not inhibit FtsZ-cp proteins in accordance with the present invention. In another embodiment, tobacco FtsZ-cp protein can be used. In yet another embodiment, *Arabidopsis* FtsZ-cp protein can be used.

Embodiments of the present invention provide an antimicrobial which inhibits growth of an oomycete, wherein the antimicrobial affects the FtsZ-mt protein of the oomycete. Embodiments of the present invention provide methods of treating a fish infected with an oomycete pathogen by application of a compound identified by methods of the present invention for identifying compounds having antimicrobial activity, which methods can include the steps of combining a polypeptide having substantial similarity to at least a portion of a an oomycete FtsZ-mt protein, with a compound to be tested for the ability to bind to an FtsZ-mt protein under conditions conducive to binding, selecting a compound thus identified as capable of binding to said FtsZ-mt protein, applying the selected compound to a microbe to test for antimicrobial activity, and then selecting compounds having antimicrobial activity. Preferably, the oomycete pathogen belongs to the genus *Saprolegnia*.

Embodiments of the present invention provide methods of treating a fish infected with an oomycete pathogen by application of a compound identified by methods of the present invention for identifying an inhibitor of FtsZ-mt activity having antimicrobial activity, by combining a FtsZ-mt protein and a compound to be tested for the ability to inhibit the activity of the FtsZ-mt protein, under conditions conducive to such inhibition, selecting a compound thus identified as capable of inhibiting said FtsZ-mt activity, applying the compound identified as capable of inhibiting said FtsZ-mt activity to a microbe to test for antimicrobial activity, and selecting compounds having antimicrobial activity. Preferably, the oomycete pathogen is of the genus *Saprolegnia*.

For purposes of summarizing the invention and the advantages achieved over the prior art, certain objects and advantages of the invention have been described above. Of course, it is to be understood that not necessarily all such objects or advantages may be achieved in accordance with any particular embodiment of the invention. Thus, for example, those skilled in the art will recognize that the invention may be embodied or carried out in a manner that achieves or optimizes one advantage or group of advantages as taught herein without necessarily achieving other objects or advantages as may be taught or suggested herein.

Further aspects, features and advantages of this invention will become apparent from the detailed description of the preferred embodiments which follow.

Brief Description of the Drawings

These and other features of this invention will now be described with reference to the drawings of preferred embodiments which are intended to illustrate and not to limit the invention.

Figure 1. Complete cDNA sequence of FtsZ-mt2 from *Phytophthora infestans*, showing nucleotide sequence (SEQ ID NO: 9) and predicted amino acid sequence (SEQ ID NO: 10).

Figure 2. Alignment of FtsZ sequences from *Agrobacterium tumefaciens* (SEQ ID NO: 11), *Sinorhizobium meliloti* (SEQ ID NO: 12), *Bartonella clarridgeiae* (SEQ ID NO: 13), *Rickettsia prowazekii* (SEQ ID NO: 14), *Caulobacter crescentus* (SEQ ID NO: 15), *Cyanidioschyzon merolae*-mt (SEQ ID NO: 16), *Phytophthora infestans*-mt2 (SEQ

0970509.01304

ID NO: 4), *Mallomonas splendens*-mt (SEQ ID NO: 17), *Phytophthora infestans*-mt1 (SEQ ID NO: 2), *Gentiana lutea*-cp (SEQ ID NO: 18), *Nicotiana tabacum*-cp2-1 (SEQ ID NO: 19), *Arabidopsis thaliana*-cp2 (SEQ ID NO: 20), *Physcomitrella patens*-cp1 (SEQ ID NO: 21), *Physcomitrella patens*-cp2 (SEQ ID NO: 22), *Guillardia theta*-cp (SEQ ID NO: 23), *Mallomonas splendens*-cp (SEQ ID NO: 24), *Anabaena* (SEQ ID NO: 25), *Synechocystis* (SEQ ID NO: 26), *Arabidopsis thaliana*-cp1 (SEQ ID NO: 27), *Pisum sativum*-cp (SEQ ID NO: 28), *Nicotiana tabacum*-cp1-3 (SEQ ID NO: 29), *Nicotiana tabacum*-cp1 (SEQ ID NO: 30), *Nicotiana tabacum*-cp1-1 (SEQ ID NO: 31), and *Nicotiana tabacum*-cp2 (SEQ ID NO: 32).

Figure 3. Phylogenetic tree of FtsZ proteins. The phylogenetic relationship among chloroplastic, cyanoobacterial, mitochondrial and proteobacterial FtsZs is shown based upon comparison of amino acid sequences. The phylogenetic tree also includes Archaea and bacteria other than Proteobacteria. Both Figure 2 and Figure 3 were constructed using a clustal method (Higgins and Sharp (1989) *Comput Appl Biosci* 5: 151-153).

Detailed Description of the Preferred Embodiment

The presently claimed invention is drawn to the use of FtsZ-mt as a target for antimicrobials. FtsZ-mt is an ideal target as it appears to be unique to primitive eukaryotes such as oomycetes and has no counterpart in true fungi and higher eukaryotes.

Preferred embodiments of the present invention will be explained below. However, the present invention includes various embodiments and is not limited to the preferred embodiments.

The invention relates to genes isolated from *Phytophthora infestans* that code for an essential protein having a proposed role in mitochondrial division (Beech *et al.* (2000) *Science* 287:1276-1279). The invention also includes the methods of using these proteins as antimicrobial targets, based on the essentiality of the gene for normal growth and development. The invention is also useful as a screening assay to identify inhibitors that are potential antimicrobials.

109770509 "043904
The use of antimicrobials to control the growth of undesirable microbes that
cause disease has become almost a universal practice. Antimicrobials against plant
disease agents may be applied to a plant, a plant part, and/or the soil surrounding a
plant. Antimicrobials against animal disease agents may be applied directly to an
5 animal, or may be applied to the environment, for example in solution to treat fish.
However, development of resistance of microbes to antimicrobials is a major problem.
Extensive use of the same antimicrobial kills susceptible individuals, while resistant
individuals survive, which permits resistant individuals to reproduce and pass on their
capacity for resistance to their progeny. The progeny take over niches formerly
10 occupied by susceptible individuals. Eventually, the whole population may become
resistant, resulting in a failure of pest management practices.

As antimicrobials are lost to the development of resistance in target populations,
the production of effective new antimicrobials becomes increasingly important. It is
important that these chemicals also be harmless to higher eukaryotes. Novel
15 antimicrobials can now be discovered using high-throughput screens that implement
recombinant DNA technology. Metabolic enzymes found to be essential to microbial
growth and development can be recombinantly produced through standard molecular
biological techniques and utilized as antimicrobial targets in screens for novel inhibitors
of protein functions. Novel inhibitors discovered through such screens may then be
20 used as antimicrobials to control undesirable microbial growth.

Virtual screens can also be used based upon the predicted three-dimensional
structure of the protein of interest. Chemicals that bind to the microbial protein, but not
to the corresponding higher plant protein, are good candidates for antimicrobials which
can then be tested for efficacy in a particular organism. Candidates for antimicrobials
25 may be tested for efficacy in a cultivation system such as a crop system or an
aquaculture system, or in a less-managed system such as of oak trees in a mixed stand.

Persistent and ongoing problems with microbial proliferation remain, causing
various plant diseases including root rot, fruit rot, mealy rot, damping off, cortical rot,
foliar blight, leaf blight, rhizome rot, root disease, white rust, and downy mildew (See
30 Table 1). Furthermore, as human populations continue to grow, there will be increasing
food shortages as food is lost to pre-harvest and post-harvest disease. Therefore, there

exists a long-felt and yet-unfulfilled need to find new, effective, and economic antimicrobials.

Definitions:

5 For clarity, certain terms used in the specification are generally defined and presented as follows:

“Chimeric” as used herein, is generally used to indicate that a nucleic acid sequence, such as a vector or a gene, is comprised of more than one nucleic acid sequence of distinct origin, where the nucleic acid sequences are fused by recombinant techniques resulting in a nucleic acid sequence which does not occur naturally.

“Expression” as used herein, generally refers to the transcription and/or translation of an endogenous gene, a transgene, or other construct capable of being transcribed and/or translated. In the case of antisense constructs, for example, expression may refer to the transcription of the antisense RNA.

15 “Gene” as used herein, generally refers to a coding sequence, optionally associated with regulatory sequences, wherein the coding sequence is normally transcribed into RNA such as, for example, mRNA, rRNA, tRNA, snRNA, sense RNA or antisense RNA. Examples of regulatory sequences are promoter sequences, 5’ and 3’ untranslated sequences, and the like. Further elements that may be present are, for example, introns.

20 “Antimicrobial” as used herein, generally refers to a substance, compound, or composition, whether chemical, biological, or otherwise, that is used to kill or suppress the growth or reproduction of microbes such as bacteria, molds, mildews, fungi, and oomycetes.

25 “Heterologous Sequence” as used herein, generally refers to a nucleic acid or polypeptide sequence not naturally associated with a host cell into which it is introduced, including non-naturally-occurring multiple copies of a naturally occurring nucleic acid or polypeptide sequence, where the term may also refer a nucleic acid or polypeptide sequence not naturally associated with the other nucleic acid or polypeptide sequences in a construct.

30

“Inhibitor” as used herein, generally refers to a substance, compound, or composition, whether chemical, biological, or otherwise, that inactivates the activity of an active or functional molecule or complex, such as, for example, a biosynthetic enzyme, receptor, signal transduction protein, structural gene product, or transport protein. Preferred inhibitors may disrupt activities that are essential to the growth, reproduction, or survival of a plant.

“Isolated” as used in the context of the present invention, such as an isolated DNA or protein molecule, generally refers to a molecule, substance, compound, or composition that, by the hand of man, exists apart from its native environment and is therefore not a product of nature. For example, an isolated DNA molecule or protein may exist in a purified form or may exist in a non-native environment such as, for example, a transgenic host cell.

“Mature protein” as used herein, generally refers to a protein that is subject to post-translational modifications, and that has undergone one or more such modifications. For example, a protein that is normally targeted to a cellular organelle, such as a chloroplast, from which the transit peptide has been removed, is a mature protein.

“Operably linked to” or “associated with” as used herein, refer generally to two or more nucleic acid sequences that are functionally juxtaposed or adjacent. For example, a regulatory DNA sequence is said to be “operably linked to” or “associated with” a DNA sequence that codes for an RNA or a protein, if the two sequences are situated such that the regulatory DNA sequence affects expression of the coding DNA sequence.

“Plant” as used herein, refers generally to any plant or plant part such as, for example, leaves, stems, roots, flowers or flower parts, fruits, pollen, pollen tubes, ovules, embryo sacs, egg cells, zygotes, embryos, seeds, cuttings, and particularly to seed plants.

“Plant cell” as used herein, refers generally to a structural and physiological unit of a plant. The plant cell may be in form of an isolated single cell or a cultured cell which may or may not contain a cell wall, or as a part of higher organized unit such as, for example, a plant tissue, or a plant organ.

“Plant material” as used herein, refers generally to whole plants or plant parts such as leaves, stems, roots, flowers or flower parts, fruits, pollen, pollen tubes, ovules, embryo sacs, egg cells, zygotes, embryos, seeds, cuttings, cell or tissue cultures, or any other part or product of a plant, such as rubber, agar or wood products.

5 “Region” or “Portion” of a nucleic acid or polypeptide sequence, as used herein, refers generally to a substantially contiguous segment of adjacent nucleic acids or amino acids in a nucleic acid or polypeptide sequence, such that the segment is of sufficient length to encode or include a functional or active part or domain of a polypeptide, or a plurality of such functional or active domains, whether or not such domains are
10 separated by other sequences. As an alternative, a region or portion can encode or include a part of a polypeptide that is not functional or active from the perspective of a native protein, but which carries at least one other kind of useful feature, such as, for example, an epitope that is recognized by an antibody, a structure that is recognized by an inhibitor, and the like.

15 “Selectable marker” as used herein, refers generally to a sequence whose expression permits selection of a cell expressing the marker, or differentiation between a cell expressing or containing the marker and a cell that does not express or contain the marker. A preferred selectable marker generally confers a selective advantage on the cell in which it is expressed or which contains it. The selective advantage possessed by
20 transformed cells having the selectable marker may be due to their ability to grow in the presence of a negative selective agent, such as an antibiotic or an antimicrobial, compared to the growth of non-transformed cells. The selective advantage possessed by the transformed cells, compared to non-transformed cells, may also be due to their enhanced or novel capacity to utilize an added compound as a nutrient, growth factor or
25 energy source. “Selectable marker gene” can also refer to a gene or a combination of genes whose expression in a cell gives the cell both a negative and a positive selective advantage.

 “Sequence Identity” refers generally to a quantitative measure of the degree to which two or more sequences are identical. The percentage of sequence identity is
30 typically determined using computer programs that are based on dynamic programming algorithms. Computer programs that are preferred within the scope of the present

invention include the BLAST (Basic Local Alignment Search Tool) search programs designed to explore all of the available sequence databases regardless of whether the query is protein or DNA. Version BLAST 2.0 (Gapped BLAST) of this search tool has been made publicly available on the Internet (currently available at
5 <http://www.ncbi.nlm.nih.gov/BLAST/>). It uses an heuristic algorithm which seeks local as opposed to global alignments and is therefore able to detect relationships among sequences which share only isolated regions. The scores assigned in a BLAST search have a well-defined statistical significance.

The term “substantially similar” as used herein, generally refers to the
10 correspondence of a given nucleotide or polypeptide sequence to a reference sequence. A nucleic acid sequence that is substantially similar to a reference sequence may encode a polypeptide having substantially the same structure and function as the polypeptide encoded by the reference nucleotide sequence, preferably, for example, where the only changes in amino acids do not affect the polypeptide function. Desirably the
15 substantially similar nucleotide sequence encodes the polypeptide encoded by the reference nucleotide sequence. The term “substantially similar” is specifically intended to include nucleotide sequences wherein the sequence has been modified to optimize expression in particular cells. The percentage of identity between the substantially similar nucleotide sequence and the reference nucleotide sequence desirably is at least
20 about 65%, more desirably at least about 75%, preferably at least about 85%, more preferably at least about 90%, still more preferably at least about 95%, yet still more preferably at least about 99%.

A nucleotide sequence “substantially similar” to a reference nucleotide sequence hybridizes to the reference nucleotide sequence in 7% sodium dodecyl sulfate (SDS),
25 0.5 M NaPO₄, 1 mM EDTA at 50°C with washing in 2X SSC, 0.1 % SDS at 50°C; more desirably hybridization is in 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO₄, 1 mM EDTA at 50°C with washing in 1 X SSC, 0.1 % SDS at 50°C; more desirably still, hybridization is in 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO₄, 1 mM EDTA at
30 50°C with washing in 0.5X SSC, 0.1% SDS at 50°C; more preferably, hybridization is in 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO₄, 1 mM EDTA at 50°C with washing in 0.1 X SSC, 0.1 % SDS at 50°C; more preferably, hybridization is in 7% sodium

dodecyl sulfate (SDS), 0.5 M NaPO₄, 1 mM EDTA at 50°C with washing in 0.1 X SSC, 0.1 % SDS at 65°C.

“Transformation” as used herein, generally refers to a process for introducing one or more heterologous nucleic acids into a cell, tissue, or plant. Transformed cells, tissues, or plants are understood to encompass not only the end product of a transformation process, but also transgenic progeny thereof.

“Transgenic” as used herein, refers to an organism containing a cell or cells that are transformed with, for example, a recombinant DNA molecule that preferably comprises a suitable promoter operatively linked to a DNA sequence of interest.

One object of the present invention is to provide essential genes in oomycetes for use in screening assays for inhibitory compounds having antimicrobial activity. Both FtsZ-mt and FtsZ-cp are nuclear-encoded. The protein products are transported into mitochondria and chloroplasts, respectively, by an N-terminal signal peptide. The FtsZ-mt gene is thought to be essential for mitochondrial division in oomycetes but has relatively low sequence identity to the corresponding chloroplast gene, FtsZ-cp (see Figure 3). This indicates that some chemicals which inhibit the function of the FtsZ protein in oomycetes can have detrimental effects on oomycetes, but little or no effect on their plant hosts, and thus are potentially good antimicrobial candidates. FtsZ-mt also has no counterpart in higher eukaryotes such as mammals. Thus, selected chemicals with activity against FtsZ-mt will be harmless to major groups of higher eukaryotes, and toxicity to humans is expected to be non-existent. On the other hand, chemicals with activity against oomycetes would be expected to have activity against other microbes such as α -proteobacteria, due to conservation between genes encoding FtsZ in these organisms. The present invention therefore provides methods of using a purified protein encoded by the gene sequences described herein to identify inhibitors thereof, which can then be used as antimicrobials to suppress the growth of oomycetes such as *Phytophthora*, especially *Phytophthora infestans*, as well as α -proteobacteria such as *Agrobacterium* species, on plants.

Another object of the present invention is to provide methods of crop improvement using compounds identified as having antimicrobial activity. Antimicrobials to suppress the growth of oomycetes and α -proteobacteria may be

09770509 "012301"
FO920 6052260

applied to a plant, plant part, plant seed, or surrounding soil. Methods for applying compounds identified as having antimicrobial activity in accordance with methods of the present invention may include foliar application of these compounds in a suitable mixture, application to roots in the form of a root drench or root dip, introduction of these compounds directly into plants as in injection under bark or into xylem, introduction via grafting or cut surfaces, or application in fields where crops are grown. Aspects of the present invention include treatment of a wide variety of plants, particularly agronomically important crops including, but not limited to, potato, tomato, tobacco, oaks, coffee berry (*Rhamnus* sp.), squash, cotton, sorghum, peas, onion, melon, cucumber, peas, beets, watermelon, peppers, Port Orford Cedar, taro, apple, *Brassica* species, sweet potato (*Ipomea*), spinach, beans, grapevine, sunflower, hops, lettuce, violets, asters, soybeans, and cereals including maize, rice, barley, wheat, and rye, and the like.

In an alternate embodiment, the identified inhibitors may also be used against fish pathogens such as the oomycete *Saprolegnia*, as well as α -proteobacterial species which are disease agents for animals, including humans. An advantage of the present invention is that the newly discovered essential genes whose products serve as targets of a novel antimicrobial mode of action enables one skilled in the art to easily and rapidly identify novel antimicrobials. Another advantage of the present invention is that compounds identified as having antimicrobial activity in accordance with the methods of the present invention will have no effect on higher eukaryotes treated with the compounds.

In one embodiment, the present invention provides novel polynucleotides having the sequences of SEQ ID NOS: 1, 3, 5, or 9. The nucleotide sequence of SEQ ID NO: 1 encodes a protein designated as FtsZ-mt1 having the deduced amino acid sequence of SEQ ID NO: 2. Also provided is a second novel polynucleotide having the sequence shown in SEQ ID NO: 5 which encodes a second FtsZ-mt protein designated as FtsZ-mt2 (genomic) and having the amino acid sequence of SEQ ID NO: 4. FtsZ-mt2 (genomic) encodes a partial genomic sequence which has two introns as determined by the "GT-AG" rule and by amino acid sequence homology to other FtsZ proteins. The corresponding cDNA sequence for FtsZ-mt2 (genomic) is shown in SEQ ID NO: 3.

The complete cDNA sequence corresponding to the partial genomic sequence FtsZ-mt2 is shown in SEQ ID NO: 9.

The present invention also encompasses nucleotide sequences substantially similar to those set forth in SEQ ID NOS: 1, 3, 5, or 9, wherein said nucleotide sequence is an oomycete, bacterial, or primitive eukaryote such as protistan, nucleotide sequence. Preferred is a nucleotide sequence substantially similar to those set forth in SEQ ID NOS: 1, 3, 5, or 9, wherein said nucleotide sequence is a *Phytophthora infestans* nucleotide sequence.

Further encompassed is a polynucleotide having a nucleotide sequence substantially similar to those set forth in SEQ ID NOS: 1, 3, 5, or 9, wherein the encoded protein has FtsZ activity. Particularly preferred is a nucleotide sequence substantially similar to those set forth in SEQ ID NOS: 1, 3, 5, or 9 wherein said encoded protein has FtsZ activity. Further encompassed is an amino acid sequence including an amino acid sequence encoded by a nucleotide sequence substantially similar to SEQ ID NOS: 1, 3, 5, or 9. Also encompassed is an amino acid sequence including an amino acid sequence encoded by SEQ ID NOS: 1, 3, 5, or 9.

Also encompassed are polynucleotides with substantial sequence identity to any of SEQ ID NOS: 1, 3, 5, or 9, more preferably at least about 65% sequence identity, yet more preferably at least about 70% sequence identity, yet more preferably at least about 75% sequence identity, yet more preferably at least about 80% sequence identity, yet more preferably at least about 85% sequence identity, yet more preferably at least about 90% sequence identity, yet more preferably at least about 95% sequence identity, yet more preferably at least about 99% sequence identity to any one of SEQ ID NOS: 1, 3, or 5.

The present invention also encompasses proteins whose amino acid sequences are substantially similar to the amino acid sequences set forth in SEQ ID NOS: 2, 4 or 10. Also encompassed is an amino acid sequence including an amino acid sequence substantially similar to any of SEQ ID NOS: 2, 4 or 10. Preferred is an amino acid sequence including an amino acid sequence which is SEQ ID NO: 2 or 4 or 10.

Encompassed herein is an amino acid sequence including an amino acid sequence encoded by a nucleotide sequence substantially similar to any of SEQ ID

NOS: 1, 3, 5, or 9, wherein the protein has FtsZ activity. Further encompassed is an amino acid sequence including at least 20 consecutive amino acid residues of the amino acid sequence of any of SEQ ID NOS: 2, 4 or 10.

5 A further embodiment is a chimeric gene comprising a promoter operably linked to a nucleotide sequence substantially similar to any of SEQ ID NOS: 1, 3, 5, or 9. Further encompassed is a recombinant vector including a chimeric gene comprising a promoter operably linked to a nucleotide sequence substantially similar to any of SEQ ID NOS: 1, 3, 5, or 9, wherein said vector is capable of being stably transformed into a host cell. Further encompassed is a host cell including a vector comprising a chimeric
10 gene comprising a promoter operatively linked to a nucleotide sequence substantially similar to any of SEQ ID NOS: 1, 3, 5, or 9, wherein said vector is capable of being stably transformed into a host cell and wherein said nucleotide sequence is expressible in said cell.

15 A preferred host cell according to the invention is either a prokaryotic or a eukaryotic cell. Most preferred is a host cell which is an *Escherichia coli* (*E. coli*), a yeast, or an insect cell.

In another embodiment, a novel polynucleotide having the sequence of SEQ ID NOS: 1, 3, 5, or 9 is overexpressed or underexpressed in an oomycete and effects on cell division, growth, nutrient uptake, reproduction, etc are measured.

20 Further embodied is a process of identifying compounds having antimicrobial activity including:

a) combining a protein comprising an amino acid sequence encoded by a nucleotide sequence substantially similar to a nucleotide sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 and SEQ ID NO: 9
25 and a compound to be tested for the ability to bind to said protein, under conditions conducive to binding,

b) selecting a compound identified in step (a) that is capable of binding said protein,

c) applying the compound identified compound in step (b) to an oomycete or
30 other microbe to test for antimicrobial activity, and

d) selecting compounds having antimicrobial activity.

Further encompassed is a compound having antimicrobial activity identifiable by a process according to the invention. Further encompassed is a process of identifying an inhibitor of FtsZ activity having antimicrobial activity including:

5 a) combining a FtsZ-mt protein and a compound to be tested for the ability to inhibit the activity of said FtsZ-mt protein, under conditions conducive to such inhibition,

b) selecting a compound identified in step (a) that is capable of inhibiting said FtsZ-mt protein activity,

10 c) applying the compound identified in step (b) to a microbe such as an oomycete or α -proteobacterium to test for antimicrobial activity, and

d) selecting compounds having antimicrobial activity.

15 In yet another embodiment, expression of the novel polynucleotide having the sequence of SEQ ID NOS: 1, 3, 5, or 9 in an oomycete is decreased and effects on cell division, growth, nutrient uptake, reproduction, etc is measured. Any inhibitor of FtsZ activity has potential as a fungicide for control of oomycete infection in higher plants.

20 A further embodiment of the invention is a compound having antimicrobial activity identifiable by a process according to the invention. Further encompassed is a method for suppressing the growth of an oomycete comprising, applying to said oomycete a compound that inhibits the activity of the amino acid sequence comprising an amino acid sequence encoded by a nucleotide sequence substantially similar to SEQ ID NOS: 1, 3, 5, or 9 in an amount sufficient to suppress the growth of said oomycete. In a preferred embodiment, the inhibiting compound is applied to *Phytophthora infestans*.

25 Further encompassed is a process of identifying compounds having antimicrobial activity including:

a) combining a protein selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4 and SEQ ID NO: 10 and a compound to be tested for the ability to bind to said protein, under conditions conducive to binding,

30 b) selecting a compound identified in step (a) that is capable of binding said protein,

c) applying a compound identified in step (b) to a microbe such as an oomycete to test for antimicrobial activity, and

d) selecting compounds having antimicrobial activity.

Further encompassed is a method of improving crops including, applying to a plant, plant part, plant seed, or surrounding soil a compound according to the invention having antimicrobial activity in an amount that inhibits the growth of undesirable microbes without significantly suppressing the growth of the plant or seed.

Furthermore, the invention can be used in a screening assay to identify inhibitors that are potential antimicrobials. Further embodied is a method which employs a virtual screen of inhibitors of the FtsZ function to be tested, including

a) determining the 3-dimensional structure of an FtsZ-mt protein using a computer model,

b) determining the 3-dimensional structure of an FtsZ-cp protein using a computer model, and

c) testing chemicals for their ability to bind to FtsZ-mt, and not to FtsZ-cp, using a computer model.

The invention also provides methods for suppressing the growth of an oomycete or an α -proteobacterium including the step of applying to the oomycete or the α -proteobacterium a chemical that inhibits the naturally occurring FtsZ-mt activity in the oomycete or α -proteobacterium. In a related aspect, the present invention is directed to a method for selectively suppressing the growth of plant pests including, but not limited to, microbes such as those from the genera *Agrobacterium*, *Lagen*, *Peronophythora*, *Trachysphaera*, *Pythium*, *Phytophthora*, *Albugo*, *Peronospora*, *Plasmopora*, *Pseudoperonospora*, *Bremia*, *Bremiella* and *Basidiophora*, in a field containing a crop of planted crop seeds or plants, including the steps of:

a) optionally planting crops or crop; and

b) applying to the crop plant, plant part, plant seed, or surrounding soil in the field an antimicrobial in amounts that inhibit naturally occurring FtsZ-mt activity in an oomycete or an α -proteobacterium without affecting FtsZ-cp activity in the plant plastid, wherein the antimicrobial suppresses the growth of microbes such as those from the genera *Lagen*, *Peronophythora*, *Trachysphaera*, *Pythium*, *Phytophthora*, *Albugo*,

Peronospora, Plasmopora, Pseudoperonospora, Bremia, Bremiella and Basidiophora without significantly suppressing the growth of the crops.

A further aspect of the invention provides means to treat plant species including, but not limited to, potato, tomato, tobacco, oaks, coffee berry (*Rhamnus* sp.), squash, cotton, sorghum, peas, onion, melon, cucumber, peas, beets, watermelon, peppers, Port Orford Cedar, taro, apple, *Brassica* species, sweet potato (*Ipomea*), spinach, beans, grapevine, sunflower, hops, lettuce, violets, asters, soybeans, and cereals including maize, rice, barley, wheat, and rye, and the like.

In an alternate embodiment, the present invention provides means to treat fish infected with an oomycete, such as an oomycete from the genus *Saprolegnia*. The infected fish may be treated with a chemical that inhibits the naturally occurring FtsZ-mt activity in the oomycete. A fish may be treated by direct external or internal application of a compound identified by methods of the present invention, and/or may be treated by exposure to a compound identified by methods of the present invention in solution.

Recombinant Production of FtsZ and Uses Thereof. For recombinant production of FtsZ in a host organism, a nucleotide sequence encoding FtsZ protein is inserted into an expression cassette designed for the chosen host and introduced into the host, where it is recombinantly produced. The choice of specific regulatory sequences such as promoter, signal sequence, 5' and 3' untranslated sequences, and enhancer appropriate for the chosen host is within the scope of knowledge of one of skill in the art. The resultant molecule, containing the individual elements operably linked in proper reading frame, may be inserted into a vector capable of being transformed into the host cell. Suitable expression vectors and methods for recombinant production of proteins are well known for host organisms such as *E. coli*, yeast, and insect cells (see, e.g., Luckow and Summers (1988) *BioTechnol* 6: 47) and baculovirus expression vectors, e.g., those derived from the genome of *Autographa californica* nuclear polyhedrosis virus (AcMNPV).

In a preferred embodiment, the nucleotide sequence encoding a protein having FtsZ-mt activity is derived from an oomycete and most preferably from *P. infestans*. In a further preferred embodiment, the nucleotide sequence is identical or substantially

similar to the nucleotide sequence set forth in any of SEQ ID NOS: 1, 3, 5, or 9, or encodes a protein having FtsZ-mt activity, whose amino acid sequence is identical or substantially similar to the amino acid sequence set forth in any of SEQ ID NOS: 2, 4 or 10.

5 The nucleotide sequence set forth in SEQ ID NO: 1 is a genomic sequence which encodes a *Phytophthora infestans* FtsZ-mt1 protein, whose amino acid sequence is set forth in SEQ ID NO:2. The genomic nucleotide sequence set forth in SEQ ID NO: 5 also encodes a FtsZ-mt2 protein containing 2 introns as determined by the “GT-AG” rule and amino acid sequence homology to known FtsZ proteins. The
10 corresponding cDNA is set forth in SEQ ID NO: 3. SEQ ID NO: 9 shows the sequence of a full length cDNA corresponding to the FtsZ-mt2 genomic sequence. The corresponding protein sequence is shown as SEQ ID NO: 10. The FtsZ-mt2 protein (SEQ ID NO: 10) is predicted by TargetP (Emanuelsson *et al.* (2000) *J. Mol. Biol* 300:1005-1016) to be transported into the mitochondrion.

15 Recombinantly produced FtsZ-mt is isolated and purified using a variety of standard techniques. The actual techniques that may be used will vary depending upon the host organism used (see, *e.g.*, chapter 16 of Ausubel, F. *et al.*, “Current Protocols in Molecular Biology”, pub. by John Wiley & Sons, Inc. (1994)).

Screening of Random Chemicals and Known Antimicrobials.

20 Recombinantly produced FtsZ-mt proteins are useful for a variety of purposes. For example, they can be used in *in vitro* assays to screen known antimicrobial chemicals whose target has not been identified, to determine if they inhibit FtsZ-mt. Such *in vitro* assays may also be used as more general screens to identify chemicals that inhibit FtsZ protein function and that are therefore novel antimicrobial candidates. Alternatively,
25 recombinantly produced FtsZ proteins may be used to elucidate the complex structure of these molecules and to further characterize their association with known inhibitors in order to rationally design new inhibitory antimicrobials as well as antimicrobial-tolerant forms of the enzymes.

30 Nucleotide sequences substantially similar to any of SEQ ID NOS: 1, 3, 5, or 9 and proteins substantially similar to SEQ ID NOS :2, 4 or 10 from any source, including microbial sources, can be used in the assays exemplified herein. Desirably, such

nucleotide sequences and proteins are derived from oomycetes. More desirably, they are derived from *P. infestans*.

Novel technologies are being examined that can detect interactions between a protein and a ligand without regard to the biological function of the protein. A short description of three methods is presented, including fluorescence correlation spectroscopy, surface-enhanced laser desorption/ionization, and biacore technologies. Many more of these methods are currently being discovered, and some may be amenable to automated, large scale screening in light of this disclosure.

Fluorescence Correlation Spectroscopy (FCS) theory was developed in 1972 but only in recent years has the technology to perform FCS become available (Madge *et al.* (1972) *Phys Rev Lett*, 29: 705-708; Maiti *et al.* (1997) *Proc Natl Acad Sci USA*, 94: 11753-11757). FCS measures the average diffusion rate of a fluorescent molecule within a small sample volume. The sample size can be as low as 103 fluorescent molecules and the sample volume as low as the cytoplasm of a single bacterium. The diffusion rate is a function of the mass of the molecule and decreases as the mass increases. FCS can therefore be applied to protein-ligand interaction analysis by measuring the change in mass and therefore in diffusion rate of a molecule upon binding.

Surface-Enhanced Laser Desorption/Ionization (SELDI) was invented by Hutchens and Yip during the late 1980's (Hutchens and Yip (1993) *Rapid Comm Mass Spectrom* 7: 576-580). When coupled to a time-of-flight mass spectrometer (TOF), SELDI provides a means to rapidly analyze molecules retained on a chip. It can be applied to ligand-protein interaction analysis by covalently binding the target protein on the chip and analysis by MS of the small molecules retained by this protein (Worrall *et al.* (1998) *Anal Biochem* 70: 750- 756).

Biacore relies on changes in the refractive index at the surface layer upon binding of a ligand to a protein immobilized on the layer. In this system, a collection of small ligands is injected sequentially in a 2-5 microliter cell with the immobilized protein. Binding is detected by surface plasmon resonance (SPR) by recording laser light refracting from the surface. In general, the refractive index change for a given change of mass concentration at the surface layer is practically the same for all proteins

and peptides, allowing a single method to be applicable for any protein (Liedberg *et al.* (1983) *Sensors Actuators* 4: 299-304; Malmquist (1993) *Nature*, 361: 186-187).

Virtual Screens. Virtual screens are also possible based upon the structure of the proteins to be tested or upon the 3-dimensional structures determined by crystallography of recombinantly produced FtsZ-mt proteins. Alternatively, the 3-dimensional structures of FtsZ-mt proteins can be modeled on the computer utilizing the known 3-dimensional structure of the archaeon FtsZ. The model can then be used to virtually screen for chemicals which bind to the FtsZ-mts of the present invention. Chemicals which react at or near interaction surfaces are particularly good candidates because FtsZ proteins are known to interact with other protein molecules, including other FtsZ protein molecules, as part of their normal function during cell division.

Chemicals which react with the three-dimensional FtsZ-mt model will be further tested against a model of a FtsZ-cp protein. In order to be a good candidate for a specific microbial inhibitor, the test chemical must not bind the FtsZ-cp protein, in order to provide that there is no potential inhibition of plant growth. Such chemicals are also potential candidates as target-specific antimicrobials against pathogens in other higher eukaryotes including animals and humans.

Chemicals which bind to the FtsZ-mt model but not to the FtsZ-cp model are then selected as good candidates for potential specific inhibitors of oomycetes.

Potential targeting sites may be determined empirically and by any means known in the art. One strategy is to pinpoint sites which differ between oomycete mitochondrial forms of FtsZ and higher plant chloroplast forms of FtsZ. Figure 2 shows a comparison of the FtsZ-mt1 and FtsZ-mt2 protein sequences with other known FtsZ proteins. Differences between clusters of similar sequences can be determined by comparison of sequence alignments. Potential targeting sites are found at the following positions in the protein sequence: amino acid position 18, 30, 31, 62, 135, 142, 156-157, 159, 163, 189-190, 198, 210, 217, 223, 227, 236, 245, 251-252, 266, 271, 276, 287-289, 300, 302, and 306 (Figure 2). These sites show differences in FtsZ protein sequences between the cluster for mitochondria from lower eukaryotes and an α -proteobacteria, and the cluster for higher plant chloroplasts and cyanobacteria. Consequently, these

sites represent a preferred embodiment for targeting with potential inhibitors of FtsZ-mt function.

5 ***In vivo* Inhibitor Assay.** In one embodiment, a suspected antimicrobial, for example identified by *in vitro* or virtual screening, is applied to a microbe, preferably an oomycete, more preferably to *P. infestans* at various concentrations. After application of the suspected antimicrobial its effect, for example death or suppression of growth, is recorded.

Compounds active against oomycete FtsZ can be used to control the oomycete pathogens listed and combat the oomycete-caused diseases listed in Table I.

09770509 013604
FOOT 6050260

Table 1. Oomycete Pathogens of Economically Important Plant Species

PATHOGEN	REPRESENTATIVE PLANT HOST(S)	REPRESENTATIVE DISEASES
Order Pythiales		
<i>Lagenia radiculicola</i>	<i>Hordeum vulgare</i> (barley), <i>Triticum aestivum</i> (wheat), <i>Secale cereale</i> (rye)	Browning root rot
<i>Peronophythora litchii</i>	<i>Litchi</i> species (litchi)	Blossom blight, fruit rot
<i>Trachysphaera fructigena</i>	<i>Rhamnus californica</i> (coffee berry)	Mealy rot
<i>Pythium aphanidermatum</i>	<i>Cucurbita</i> species (squash), <i>Gossypium</i> species (cotton), <i>Glycine max</i> (soybean), <i>Nicotiana tabacum</i> (tobacco), <i>Lycopersicon</i> <i>esculentum</i> (tomato)	Damping off, root rot
<i>Pythium arrhenomanes</i>	<i>Zea mays</i> (corn), <i>Sorghum bicolor</i> (sorghum)	Damping off, root rot
<i>Pythium mamillatum</i>	<i>Pisum</i> species (peas), <i>Allium</i> species (onions), <i>Gossypium</i> species (cotton), <i>Cucumis</i> species (melons and cucumbers), <i>Zea mays</i> (corn)	Damping off, root rot
<i>Pythium splendens</i>	<i>Zea mays</i> (corn), and others	Cortical rot
<i>Pythium ultimum</i>	<i>Pisum</i> species (peas), <i>Beta</i> species (beets), <i>Citrullus lantus</i> (watermelon), <i>Glycine max</i> (soybean)	Damping off, root rot, fruit rot
<i>Phytophthora infestans</i>	<i>Solanum tuberosum</i> (potato)	Foliar blight

PATHOGEN	REPRESENTATIVE PLANT HOST(S)	REPRESENTATIVE DISEASES
<i>Phytophthora capsici</i>	<i>Capsicum</i> species (peppers), <i>Citrullus lanatus</i> (watermelon), <i>Cucumis</i> species (melons and cucumbers)	Fruit rots
<i>Phytophthora lateralis</i>	<i>Chamaecyparis lawsoniana</i> (Port Orford Cedar)	Root disease
<i>Phytophthora colocasiae</i>	<i>Colocasia esculenta</i> (taro)	Leaf blight, rhizome rot
<i>Phytophthora syringae</i>	<i>Malus</i> species (apple)	Fruit rot
Order Peronosporales		
<i>Albugo candida</i>	<i>Brassica</i> species (cruciferous plants)	White rust
<i>Albugo ipomoeae-</i> <i>panduratae</i>	<i>Ipomoea batatas</i> (sweet potato)	White rust
<i>Peronospora destructor</i>	<i>Allium cepa</i> (onion)	Downy mildew
<i>Peronospora farinosa</i>	<i>Beta</i> species (beets), <i>Spinacia oleracea</i> (spinach)	Downy mildew
<i>Peronospora hyoscyami</i>	<i>Nicotiana tobacum</i> (tobacco)	Downy mildew
<i>Peronospora manshurica</i>	<i>Glycine max</i> (soybean), <i>Pisum</i> species (peas), <i>Phaseolus</i> species (beans), <i>Vigna</i> species (beans)	Downy mildew
<i>Plasmopora viticola</i>	<i>Vitis vinifera</i> (grapevine)	Downy mildew
<i>Plasmopora halstedii</i>	<i>Helianthus</i> species (sunflower)	Downy mildew
<i>Pseudoperonospora</i> <i>cubensis</i>	<i>Cucurbita</i> species (squash)	Downy mildew
<i>Pseudoperonospora humuli</i>	<i>Humulus lupulus</i> (hops)	Downy mildew
<i>Bremia lactucae</i> f. sp. <i>Lactucae</i>	<i>Lactuca sativa</i> (lettuce)	Downy mildew
<i>Bremiella megasperma</i>	<i>Viola</i> species (violets)	Downy mildew
<i>Basidiophora entospora</i>	<i>Aster</i> species (asters)	Downy mildew

EXAMPLES

5 Example 1: Isolation of genomic FtsZ sequences from *Phytophthora infestans*

FtsZ-mt1 (SEQ ID NO: 1) and FtsZ-mt2 (genomic) (SEQ ID NO: 5) were obtained from *P. infestans* genomic sequences by degenerate PCR using Platinum Taq (LTI, for hot start PCR) using the reaction conditions recommended by manufacturer (LTI). A 30 µl reaction contained 20 ng of *P. infestans* genomic DNA and 15 pmol each of the primers. One of two combinations of the primers (PI01A & PI03B or PI01A & PI06B; primers shown below) were used. The letter "T" in the primers shown below indicates that inosine is used at that site. The letter "Y" in primer PI01A (SEQ ID NO: 6) is used to indicate that T or C (a pyrimidine base) may be used at that site. The letter "R" in primer PI06B (SEQ ID NO: 8) is used to indicate that G or A (a purine base) may be used at that site.

PI01A

5'-AAYGCGTIAAAYAAYATGAT-3' (SEQ ID NO: 6)

PI03B

5'-GTICCGTICCCICCCCAT-3' (SEQ ID NO: 7)

20 PI06B

5'-GTICKIACRTCIGCRAARTC-3' (SEQ ID NO: 8)

The PCR program was:

1) 94°C, 3 min.

2) 94°C, 15 sec

25 3) 52°C, 25 sec

4) 72°C, 45 sec

Steps 2-4 were repeated 34 times (total 35 thermal cycles)

5) 72°C, 15 min.

The annealing temperature used in step 3 was determined based on a preliminary temperature gradient experiment.

30

The PCR products were separated by agarose gel electrophoresis. Two products of 250 bp (for FtsZ-mt1) and 420 bp (FtsZ-mt2 (genomic)) from the reaction with the primer combination of PI01A (SEQ ID NO: 6) and PI03B (SEQ ID NO: 7), and one product of 600bp (FtsZ-mt1) from the reaction with the primer combination of PI01A (SEQ ID NO: 6) and PI06B (SEQ ID NO: 8) were isolated from the gel.

The isolated PCR products were cloned into PCR2.1-TOPO vector using TOPO-TA cloning kit (Invitrogen) and the sequence of the insert sequences was determined. FtsZ-mt1 (SEQ ID NO: 1) does not have an intron. The deduced amino acid sequence for FtsZ-mt1 is shown in SEQ ID NO: 2. FtsZ-mt2 (genomic) (SEQ ID NO: 5) has two introns and the FtsZ-mt2 (genomic) cDNA (SEQ ID NO: 3) is the sequence artificially spliced based on the "GT-AG" rule and the amino acid sequence homology. The deduced amino acid sequence for FtsZ-mt2 (genomic) is shown in SEQ ID NO: 4.

Example 2: Isolation of a full length FtsZ-mt2 cDNA from *Phytophthora infestans*

A full length FtsZ-mt2 cDNA was isolated by searching a *P. infestans* EST sequence library prepared by Novartis Agribusiness Biotechnology Research, Inc.(NABRI). Clones identified by screening with FtsZ-specific probes were isolated, and the inserts of EST clones positive for FtsZ-mt2 were sequenced. FtsZ-mt2 (cDNA) (SEQ ID NO: 9) represents the complete cDNA sequence corresponding the FtsZ-mt2 (genomic) cDNA (SEQ ID NO: 3). The deduced amino acid sequence for FtsZ-mt2 (cDNA) is shown in SEQ ID NO: 10.

Example 3: Screening methods for inhibitors of FtsZ: Computer modeling

The 3-dimensional structure of FtsZ-mt1 and FtsZ-mt2 (genomic and cDNA) and the chloroplast FtsZ (FtsZ-cp) will be determined by computer using a software program such as MolSoft™. Chemicals will be virtually screened to look for those which can bind to FtsZ-mt, but not to FtsZ-cp. Any chemicals which are capable of binding to FtsZ-mt, but not to FtsZ-cp are good candidates for specific inhibitors of FtsZ-mt and have potential as antimicrobial agents in methods of pest control. In addition, FtsZ proteins are known to interact with other proteins as part of their normal

function during cell division. Consequently, chemicals that bind at or near interaction surfaces will make especially good candidates as potential antimicrobials.

Figure 2 shows a comparison of the FtsZ-mt1 sequence (SEQ ID NO: 1) with other known FtsZ proteins. Potential targeting sites are indicated at the following positions in the protein sequence: 18, 30, 31, 62, 135, 142, 156-157, 159, 163, 189-190, 198, 210, 217, 223, 227, 236, 245, 251-252, 266, 271, 276, 287-289, 300, 302, and 306. The indicated sites were well-clustered by homology with FtsZs from an (α)-proteobacteria and other mitochondrial forms, but well-separated from the cluster for the chloroplast forms and cyanobacterial FtsZs. Chemicals will be designed that can discriminate and recognize one of the amino acids in a given cluster. Such chemicals will only kill primitive eukaryotes having FtsZ-mt proteins, such as oomycetes, and some bacteria that have highly homologous FtsZ proteins, such as an α -proteobacterium, but will not affect other eukaryotes, including plants and animals.

The indicated residues were well conserved within the clusters which indicated a constraint to conserve the residue within this branch of phylogeny. Due to the constraint, if a chemical targets one of the residues listed above, it is likely that the oomycete pathogen will not easily develop resistance to that chemical because mutations in these positions would likely cause negative effects. Also, other oomycetes will also have the conserved residue. Thus, the chemical will be effective on a wide variety of microbial pathogens, not just *Phytophthora infestans*.

It will be understood by those of skill in the art that numerous and various modifications can be made without departing from the spirit of the present invention. Therefore, it should be clearly understood that the forms of the present invention are illustrative only and are not intended to limit the scope of the present invention.